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CONTENTS

SUMMARY and CONCLUSIONS

An understanding of the complexity of cumulative risks is a prerequisite for the development of more efficient guidelines to provide data for future regulation of chemicals. For this reason it is important that we improve our understanding of complex exposure situations and develop adequate tools for assessing the cumulative risk of combined exposure. It is increasingly being recognised that such tools should take into account spatial variability, especially in a truly cumulative approach, where it is realised that ecological receptors are exposed to toxic mixtures in a heterogeneous environment.

An existing spatially explicit random walk exposure model (NoMiracle Deliverable 4.2.1) was enhanced to model exposure to multiple stressors and to calculate its cumulative effects using concentration addition and response addition principles. This Spatially explicit Cumulative Exposure model, SpaCE model, has been parameterised for nickel and applied to a cadmium and nickel exposure case in the 'Afferdensche en Deestsche Waarden' study area. The results showed that all species modelled are exposed well below the toxicity reference value for nickel, which hence does not pose a serious risk to these species. The interspecific differences in predicted nickel exposure can mainly be explained by the variations in diet preferences of the species. The intraspecific variability in predicted nickel exposure is caused by both spatially variable nickel concentrations in soil and location-specific availability of diet items. Comparison of estimated and measured internal nickel concentrations for four vertebrate species showed a systematic and substantial difference between the two, predicted values always being the lower ones. Therefore revision of the applied formulas for nickel accumulation is recommended, e.g. by explicitly incorporating absorption and excretion kinetics.

The cumulative effect results showed that cadmium was the main contributor causing adverse effects to the species modelled. In only one case, namely for the Common vole and assuming concentration additivity, was an increased effect predicted for exposure to the mixture of cadmium and nickel compared to cadmium exposure alone. Compared to single exposure, cumulative exposure practically always increased the variability of the predicted risk. Overall, the SpaCE model provides an adequate tool for predicting cumulative exposure to co-occurring contaminants in a spatially explicit and individual-based manner. This approach allows the model to predict inter-individual variability of (cumulative) exposure and the model can be applied to multiple substances with either similar or dissimilar mode of action without interaction.

1 Introduction

1.1 Background

It is generally acknowledged that chemical, biological, and other physical stressors can cause a variety of effects on human and ecological health. However, assessing the risks associated with them is, both methodologically and computationally, considerably more complex than current risk assessment practices. Exposure to contaminants involves spatially complex situations due to the heterogeneity of contaminant distributions and other environmental characteristics. An understanding of the complexity of cumulative risks (i.e. risk to chemical mixtures or multiple stressors) is a prerequisite for the development of more efficient guidelines to provide data for future regulation of chemicals. For this reason, it is important that we improve our understanding of complex exposure situations and develop adequate tools for risk assessment (NoMiracle, 2006). As a further and essential step in exposure and risk assessment it is increasingly being recognised that such tools should take into account spatial variability (Marinussen & Van der Zee 1996; Hope 2000; Korre *et al*. 2002; Linkov *et al*. 2002; Gaines *et al*. 2005; Makropoulos & Butler 2006).

When focussing on cumulative risk, spatially explicit modelling is especially important. In a truly cumulative approach it is realised that human and ecological receptors are not exposed to individual substances in a relatively homogeneous environment, but to toxic mixtures in a heterogeneous environment. The spatial component is regarded as an important contributor to variation in exposure (Kooistra *et al*., 2001; Hope, 2000; Clifford *et al*., 1995; Kareiva & Wennergren, 1995) and therefore relevant to include when predicting exposure to co-occurring stressors. At different locations, receptors are exposed to varying combinations and concentrations of multiple stressors.

Recently a spatially explicit individual-based exposure model, IBEM, has been developed, within the framework of the EU-NoMiracle project and carried out by the Radboud University Nijmegen (NoMiracle Deliverable 4.2.1), as a novel tool for ecological risk assessment (Loos *et al*., 2006). This model estimates exposure of higher terrestrial organisms to contamination, taking into account food-web relations and spatial variation associated with the exposure. The model simulates the terrestrial organisms as individual receptors by moving them over a raster map, whereby they encounter and accumulate contamination over space and time. Spatial variation is accounted for by incorporating spatially explicit contaminant concentrations and habitat use, based on habitat quality and diet requirements. The model has been applied to investigate the influence of movement and habitat use of 10 terrestrial vertebrate species on exposure to spatially variable soil cadmium contamination in a Dutch floodplain area (Loos *et al*., 2006). It proved suitable for predicting exposure to cadmium contamination and the model provides a valuable tool to generate spatially explicit exposure estimates that include intraspecific variation specifically resulting from spatially explicit behaviour.

The model approach seems also suitable to predict spatially variable exposure to other stressors. And more importantly, it seems not only suitable for predicting exposure to single substances, but to multiple stressors simultaneously (Hope, 2005). However, cumulative exposure assessment is not yet included in the IBEM model.

1.2 Objective

The aim of this study is to develop a generic model for cumulative exposure and risk assessment that addresses the spatial heterogeneity for ecological receptors and to tailor and apply such a model to a case study. Such a model is a further step towards modelling of exposure to multiple stressors. By means of combining input of multiple stressors, the IBEM model could predict location-specific integrated exposure to these stressors in a spatially explicit and receptor-oriented manner. In order to achieve this, this model has been parameterised for another heavy metal, and extended with a toxicity effects module for combined exposure, using concentration addition and response addition principles.

The extended model was applied to a food web of terrestrial vertebrate species in the Afferdensche en Deestsche Waarden (ADW), a floodplain along the river Waal, the main distributary of the river Rhine in the Netherlands. This study area was chosen because the previous model has already been parameterised for this area and for the metal cadmium. Moreover this area is polluted with other heavy metals like nickel, lead, cupper and zinc of which contamination data was readily available. The model has subsequently been parameterised for nickel making it possible to investigate the combined effects of the heavy metals cadmium and nickel. In the near future the model may be applied to a selection of other pollutants (e.g. Zn, Cu, Pb).

This report describes the individual based Spatially explicit Cumulative Exposure model (SpaCE model), that is to say, it describes those parts of the model that have changed in comparison with the previous IBEM model. For intelligibility, section 2.1 briefly introduces the previous model IBEM and section 2.2 depicts the case study. The parameterisation of the model for exposure to nickel will be discussed in section 2.3 together with the case study-specific input data for nickel and section 2.4 covers the extension of the IBEM model with the added cumulative effects module. Chapter 3 shows and discusses the results for the case study and discusses the validation of the model for nickel. Finally, the conclusions and recommendations will be given in Chapter 4.

2 Methods

This chapter first gives a brief description of the previous non-cumulative IBEM model (section 2.1). For a more detailed description of this model, please refer to Loos *et al.* (2006). The case study is discussed in section 2.2. Section 2.3 describes the modifications made to the cadmium parameterised exposure module in order to model accumulation of nickel. It also presents the case-specific nickel input data. In section 2.4, the various model improvements made to develop the cumulative IBEM model, further referred to as Spatially explicit Cumulative Exposure model (SpaCE model), are described.

2.1 Original model

Model structure

In the individual-based exposure model IBEM, the simulation is performed in a landscape divided into a regular grid with a spatial resolution of 5 by 5 meter. Each cell of the landscape grid contains information on contaminant concentrations and on environmental parameters influencing foraging behaviour (such as ecotope type and, for this specific floodplain study area, distance to flood-free terrain). An individual receptor is represented by a set of algorithms that describe the processes relevant for exposure and risk assessment, which can generally be classified into moving and uptake algorithms. Movement algorithms allow the receptor to move over a raster map, thereby encountering and accumulating contamination over space and time. Spatial variation is accounted for by incorporating spatially explicit contaminant concentrations and foraging behaviour, based on habitat and diet requirements. A so-called food web approach has been followed, which takes into account feeding relationships between species.

The exposure model is constructed in MS Excel® with the MS Visual Basic Application®. The program code contains several modules to calculate species-specific exposure, of which the most important are (1) a landscape module, (2) a foraging path module, and (3) an exposure and risk module. The *landscape module* tailors the spatial input data for the *foraging path* and the *exposure and risk modules*. In the *foraging path module*, movement algorithms allow individual receptors to move from grid cell to grid cell, thereby obeying species-specific movement rules. Subsequently, the *exposure and risk module* calculates exposure for each cell of the foraging path established in the previous module, and for the entire foraging path established. Finally, the predicted environmental concentrations (PECs), i.e. the environmental concentrations to which the organisms are exposed, are compared with the predicted no effect concentrations (PNECs) for each species to establish their level of risk.

Landscape module

The *landscape module* consists of several input maps displaying relevant environmental variables and covering the whole study area. A raster map of the contaminant concentration in soil was made by interpolation of a point database consisting of 192 cadmium concentration values measured in the study area. This was done with inverse distance weighted interpolation (IDW). The spatially explicit habitat maps in this study were created by discretising a landscape into subunits (raster cells or polygons) and calculating a habitat quality value for each unit, representing the suitability of the area on a scale ranging from 0.0 (non-habitat) to 1.0 (optimal habitat). The suitability of a site for a species is related to vegetation structure and main abiotic factors (represented by ecotopes) and its value was determined by linking an ecotope map to a species-ecotope matrix, in which each ecotope type was assigned a species-specific habitat quality value based on literature and expert knowledge. An inundation map with (distance to) flood-free terrain was made by comparing a water level, corresponding with a median discharge value leading to inundation of the study area, with a digital elevation model (DEM) of the area. Subsequently, for each grid cell the shortest distance to the thus obtained flood-free terrain was calculated.

Foraging path module

The *foraging path module*, which is only applied to mobile organisms (2nd and 3rd food web level species), consists of three parts: (1) the selection of a starting position, (2) the movement algorithm and (3) a stopping criterion. The starting position is considered as an individual's nest from which it starts to forage. For every cell in the study area, the model establishes whether the cell is a possible starting position. A cell becomes a possible starting position when it is located within suitable habitat and within a certain distance from flood-free terrain, depending on an organism's colonizing ability. Once a set of all the possible starting positions is established, one starting position is chosen randomly from this set in accordance with a distribution reflecting the species-specific colonisation probability. The simulated organism will then start foraging from this starting position. This foraging behaviour is confined to the home range area around the nest and is mainly directed by species-specific spatial variation in habitat quality, which determines the likelihood of an organism to visit this specific habitat; cells with higher habitat quality will have a higher chance of being selected as a destination cell in the foraging path, i.e. have a higher visiting probability. When the visiting probabilities are calculated, one of the cells is selected randomly from the set of selectable cells according to the distribution defined. The consecutive selection of new positions that constitute the foraging path (i.e. the foraging of the organism) stops when a stopping criterion is met, namely: until the total area foraged (i.e. the sum of the area of the grid cells visited) equals the receptor-specific foraging area within its home range.

Exposure and risk module

Cell-specific exposure concentrations are calculated in the *exposure and risk module*, using formulas that express the various routes of contaminant uptake, and depend on food web relations. The exposure of basic level organisms is governed by direct contact with the soil (e.g. through soil ingestion or dermal uptake). Their internal contaminant concentrations are calculated with bioaccumulation factors (BAFs) or regression equations. The higher level organisms are assumed to be indirectly exposed to contaminants through the intake of contaminated food. For the higher trophic level organisms, the amount of contaminant accumulated depends on their consumption rate, the assimilation efficiency of the contaminant, the age of prey consumed, and the fraction of this prey in the diet. In each cell visited, the concentration to which they are exposed is calculated and the lifetime average exposure concentration in food is determined by averaging all cell-specific concentrations of the foraging path. Finally, the predicted environmental concentrations (PECs) are compared with the predicted no effect concentration (PNECs) to determine the posed risk of the contaminant.

2.2 Case study details

For assessing the ecological risks of cumulative stressors, this case study focuses on the heavy metals cadmium and nickel. The model was applied to the same study location used for the cadmium exposure simulation, namely the embanked floodplain 'Afferdensche en Deestsche Waarden (ADW)'. Because the area is spatially heterogeneously contaminated with multiple heavy metals, of which detailed concentration distribution data is available, and because the IBEM model has already been parameterised for several organisms in this area, it is especially interesting to use the same study area for this case.

The ADW floodplain measures about 285 hectares and is located along the river Waal, which is the main distributary of the river Rhine in the Netherlands. The floodplain area between the summer and the winter dike (about two-thirds of the entire floodplain) is periodically flooded during times of high river discharge, usually once or twice a year between November and May (Wijnhoven *et al*. 2005).

During the past decades, large amounts of sediment and particulate-bound heavy metal pollution were deposited on the floodplain (Middelkoop & Asselman 1998). Because the concentrations of these heavy metals show large spatial variability in floodplain soils

(Middelkoop & Asselman 1998; Middelkoop 2000; Thonon 2006), floodplains seem ideal locations for modelling in a spatially explicit manner.

Currently, the floodplain is the subject of an ecological rehabilitation program in which safety precautions against high river discharges are combined with the conversion of agricultural land into natural floodplain ecosystems. Nature development is foreseen for almost the whole area (Ministry of V&W 2001) and hence a realistic assessment of ecological risks is highly relevant for this floodplain.

2.3 Nickel parameterisation of exposure module

2.3.1 Environmental data

Nickel concentration

A point database consisting of 181 nickel concentration values measured in the study area was compiled based on data derived from Kooistra et al. (2005) – in turn derived from five datasets (CSO 1995; Grontmij 1995; Kooistra et al., 2001; Schröder, n.d.; Kooistra et al., 2004) – and Wijnhoven *et al.* (submitted). The point data were interpolated to obtain continuous data for the whole study area. This was done with inverse distance weighted interpolation (IDW) using the Gstat software (Pebesma & Wesseling 1998). As for the other environmental variables, a spatial resolution of 5x5m was applied, resulting in a total grid of 245 rows and 912 columns (Figure 2.1).

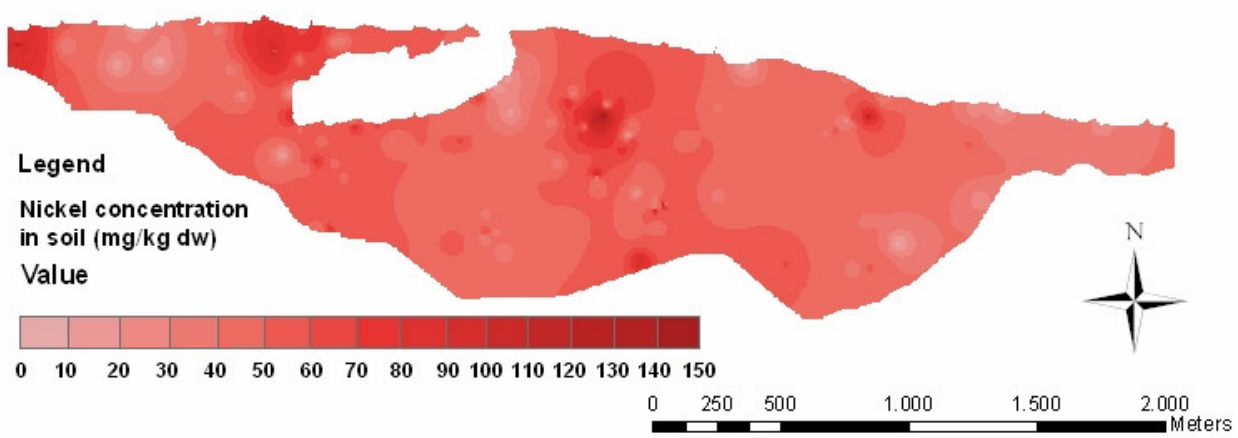


Figure 2.1 Nickel concentrations (mg/kg dw) in soil

Ecotope distribution and inundation characteristics of the study area are unchanged compared to the cadmium case study. They will not be discussed here; please refer to Loos *et al.* (2006) for details about these environmental input data.

2.3.2 Parameterisation and ecotoxicological data

This section only describes the modifications that have been made to the exposure module needed for modelling nickel accumulation as compared to cadmium accumulation. However, for a better understandability, first a short summary of cadmium exposure module is given. Please refer to Loos *et al.* (2006) for a complete description of the exposure module parameterised for cadmium.

Basically, the food web approach still holds for nickel accumulation, and only some formulas for calculating accumulation are altered together with case-specific nickel input data. This nickel input data will be described together with the corresponding accumulation formulas. Exposure and risk estimates, which are derived from lifetime exposure concentrations and risk indicators respectively, are determined for higher trophic level species (vertebrates) only and are based on contaminant concentrations in their diet. The exposure is calculated consistently with the major routes channelling the fate of the contaminant through the food web. Hereby, the organism is

exposed to the contaminant concentrations specific for the cells that form its foraging path. First, internal concentrations in 1st trophic level organisms are calculated. They are directly exposed to the cell-specific contaminant concentration in soil of the cell they live in. Secondly, cell specific internal concentrations in 2nd trophic level organisms are calculated; they are indirectly exposed to contaminants through the intake of contaminated food. From the internal concentrations of lower level organisms, the exposure concentrations in food of higher level organisms are calculated. A more detailed description of the nickel specific calculations involved in the exposure and risk assessment is given below. Note that for all equations described in this section, weight units refer to fresh weight unless indicated otherwise.

Internal concentration in 1st trophic level organisms (plants and invertebrates)

Identically to the calculation of cadmium accumulation, internal nickel concentrations for 1st trophic level, soil-dwelling and plant organisms are directly derived from soil concentrations, through the application of bioaccumulation factors (BAFs) or by means of regression equations. BAFs are empirically determined ratios of contaminant concentrations in organisms to those in soil (for nickel i.e.: $\frac{[Ni]_{organism}}{[Ni]_{soil}}$). Their application is based on the assumption that the concentration of chemicals in organisms is a linear, no-threshold function of concentrations in soil (Sample et al. 1998). However, several studies indicate that this assumption does not hold true for heavy metals, as BAFs for heavy metal concentrations in invertebrates tend to decrease with increasing soil concentrations (e.g. Gräff et al. 1997, Lock & Janssen 2001, Van Straalen et al. 2001). Log-linear regression equations are therefore likely to give more accurate results for these types of contaminants (Sample et al. 1998) and if sufficient data are available this approach should be preferred. General equations for both approaches are given below (equations 1 and 2). Both approaches yield internal concentrations in 1st trophic level organisms on a dry-weight basis.

$$^{x}\log C_{i,j,DW} = a + b \cdot {}^{x}\log(C_{i,soil;DW}) \quad (1)$$

$$C_{i,j,DW} = C_{i,soil;DW} \cdot BAF \quad (2)$$

$C_{i, j, DW}$ = contaminant concentration in prey item j in model cell i (mg·kg-1 dw)

$C_{i, soil, DW}$ = contaminant concentration in soil in model cell i (mg·kg-1 dw)

a,b = regression coefficients (dimensionless)

BAF = bioaccumulation factor (dimensionless)

It should be noted that the application of bioaccumulation factors and regression equations to determine internal concentrations of chemicals in organisms is based on the assumption of a stable ratio between a certain concentration in soil and a corresponding internal concentration in the organisms, i.e. it is assumed that the intake of the chemical is balanced by excretion and/or internal regulation mechanisms.

Table 2.1 Regression equation to calculate internal nickel concentrations (mg·kg^{-1} dw) in basic-level diet items.

	Equation	***R^2***	***n***	***P***	***Source***
Earthworms	*log [Ni-o] = -0.67 + 0.98 log [Ni-s]*	*0.66*	*180*	*<0.00001*	*Neuhauser* et al. *1995*

[Ni-o] = nickel concentration in organism (mg·kg^{-1}dw)

[Ni-s] = nickel concentration in soil (mg·kg^{-1} dw)

For the earthworms, a regression equation was selected (Table 2.1). The equation was selected based on parameters used in the equation, coefficients of determination (R^2) and significance of the relations (p).

For all other 1st trophic level species, insufficient data were available to establish regression equations and hence BAFs were selected to determine the nickel concentrations in these diet items (Table 2.2). These BAF values are calculated by taking the mean of reported BAFs, which were selected based on the sample size, the occurrence of (plant) species in the study area and excluding hyperaccumulators of nickel. For gastropods, two BAF values were needed: a BAF from soil to snails and a BAF from vegetation to snails. However, no BAF value from soil to snails was found in literature. Therefore it was calculated by multiplying the $BAF_{vegetation\text{-}to\text{-}snail}$ by the $BAF_{soil\text{-}to\text{-}vegetation}$.

Table 2.2 BAF values to calculate internal cadmium concentrations ($mg \cdot kg^{-1}$ dw) in basic-level diet items.

		BAF	***Source***
Corn	*Soil*	*0.094*	*Sadiq* et al*. 1985; Tüzen* et al*. 2003*
*Vegetation**	*Soil*	*0.058*	*Meers* et al*. 2005; Nakamura & Taira, 2005; Peterson* et al*. 2003; Robinson* et al*. 1999; Schröder* et al*. 2005*
Fruits	*Soil*	*0.008*	*INERIS 2005*
Gastropods	*Soil*	*0.003***	
	Vegetation	*0.043*	*Boyd 2002*
H&M	*Soil*	*0.031*	*Nakamura* et *al. 2005; Nakamura & Taira, 2005; Peterson* et al*. 2003*
Isopods	*Soil*	*3.503*	*Alikhan 1993; Torres & Johnson 2001*
Spiders	*Soil*	*0.024*	*Peterson* et al*. 2003; Torres & Johnson 2001*

* vegetation as diet for the selected species is assumed to consist of three vegetation types (50 % grass, 30% shrubs/herbs and 20% leaves). Calculation of the BAF for vegetation was done by summation of the BAFs for the vegetation types relative to their contribution.

** calculated by multiplying $BAF_{vegetation\text{-}to\text{-}snail}$ x $BAF_{soil\text{-}to\text{-}vegetation}$

Internal concentration in 2nd trophic level organisms (vertebrates)

Basically, the contaminant concentrations of all prey items are added, whereby the contaminant concentration in each prey item *k* present in a certain cell *i* ($C_{i,k}$) is weighted by the fraction this item represents in the diet of the receptor. It is assumed that during the foraging procedure diet fractions should always sum up to 100% and therefore the absence of a certain prey item is compensated for by proportionally enlarging the fractions of the prey items that are actually present in the cell. This reflects the assumption that the species modelled exhibit optimistic foraging behaviour. This food web approach is no different than that applied to cadmium accumulation.

However, as opposed to cadmium, nickel is an essential metal that is internally regulated (Phipps *et al*. 2002). Nickel accumulation in vertebrates can therefore not be modelled in a similar way as cadmium, for which no active metal excretion is simulated. It was chosen to model nickel accumulation in vertebrate species using a biomagnification factor (BMF), which assumes a stable ratio between a certain concentration in food and a corresponding internal concentration in the organisms. Using BMFs therefore implicitly takes into account excretion as it is assumed that the intake of the chemical is balanced by excretion and/or internal regulation mechanisms.

So the internal concentration of a 2nd level species j is dependent on the internal concentration in its diet items described by the biomagnification factor and does not depend – like cadmium – on the prey age. This relation is described for dry weight concentration and therefore the total cell-specific internal concentration needs to be converted to a fresh weight base using the species-specific dry matter content (DMC) as fraction of body total weight. This 2nd trophic level

prey item (when consumed by 3rd trophic level species) is assumed to have lived its entire life in the cell where it was caught, implicating that its internal concentration is only related to the soil contaminant concentration in this specific cell. Taking all relevant variables into account, for each 2nd trophic level prey item a cell-specific internal concentration is then calculated according to equation 3:

$$C_{i,j} = DMC_j \cdot \sum_{k=1}^{k=n} \left(BMF_{k \rightarrow j} \cdot \left(C_{i,k,DW} \cdot f_{i,k} \right) \right) \qquad (3)$$

C*i,j* = contaminant concentration in prey j in model cell i (mg·kg-1)

DMC*j* = dry matter content of prey j as fraction of fresh weight (dimensionless)

BMF*k* → *j* = bio-magnification factor from diet item k to prey j (dimensionless)

C*i,k,DW* = contaminant concentration in diet item k in model cell i (mg·kg-1)

DMC*k* = dry matter content of diet item k as fraction of fresh weight (dimensionless)

f*i,k* = fraction of diet item k in diet of prey j in model cell i (dimensionless)

n = number of diet items k

Table 2.3 BMF values to calculate internal cadmium concentrations (mg·kg^{-1} dw) in higher-level species.

Species	***Diet items***	***BMF***	***Source***
European mole	*Earthworms*	*0.076***	*Extrapolated from Common shrew data*
	H&M	*0.043***	*Extrapolated from Common shrew data*
Bank vole	*Vegetation*	*0.145*	*Bosveld* et al*. 2003*
	Fruit	*0.145*	*Bosveld* et al*. 2003*
	H&M	*0.145*	*Bosveld* et al*. 2003*
Wood mouse	*Vegetation*	*0.145**	*Extrapolated from Bank vole data*
	Fruit	*0.145**	*Extrapolated from Bank vole data*
	H&M	*0.145**	*Extrapolated from Bank vole data*
Common shrew	*Earthworms*	*0.076*	*Hendriks* et al*. 1995*
	Gastropods	*0.043*	*Bosveld* et al*. 2003; Hendriks* et al*. 1995*
	Isopods	*0.043*	*Bosveld* et al*. 2003; Hendriks* et al*. 1995*
	Spiders	*0.043*	*Bosveld* et al*. 2003; Hendriks* et al*. 1995*
	H&M	*0.043*	*Bosveld* et al*. 2003; Hendriks* et al*. 1995*
	Vegetation	*0.043*	*Bosveld* et al*. 2003; Hendriks* et al*. 1995*
Rabbit	*Vegetation*	*0.145**	*Extrapolated from Bank vole data*
Common vole	*Vegetation*	*0.145**	*Extrapolated from Bank vole data*

* These biomagnification factors are extrapolated from data on Bank voles

** These biomagnification factors are extrapolated from data on Common shrews

All BMF values are listed in Table 2.3. Only BMF values for the bank vole and the Common shrew were found in literature. Internal nickel concentrations in these species were reported as concentrations in the kidney. A ratio between nickel concentration in kidney versus nickel concentration in whole body was used to calculate the nickel concentrations in the total body of these species. These kidney-whole body ratios were calculated using data from Wijnhoven *et al*.

(submitted). The nickel concentrations in the total body, in its turn, were used to determine a BMF value for diet-to-organism transfer. In case a BMF value for soil-to-organism relation was reported, the $BMF_{diet\text{-}to\text{-}organism}$ was calculated with equation 4:

$$BMF_{diet-to-organism} = \frac{BMF_{soil-to-organism}}{BAF_{soil-to-diet}} = \frac{BMF_{soil-to-organism}}{\sum_{i=1}^{i=n}\left(BAF_{soil-to-i} \cdot f_i\right)} \qquad (4)$$

$BAF_{soil\text{-}to\text{-}i}$ = bioaccumulation factor from soil to diet item *i* (dimensionless)

f_i = fraction of diet item j (dimensionless)

For the common shrew, a BMF value was reported specific for an earthworm-to-shrew relation. This value was used and the $BMF_{diet\text{-}to\text{-}organism}$ was corrected for a diet without the earthworm. Because no nickel accumulation data was found for other 2nd trophic level species, BMF values calculated for the bank vole and common shrew were used for the other species depending on the similarity between their diets (i.e. BMF values calculated for the Common shrew were also used for the – insectivorous – European mole and BMF values calculated for the Bank vole were used for all other – herbivorous – 2nd trophic level species).

Concentration in food

Average lifetime exposure estimates are calculated in exactly the same way as for cadmium. Internal contaminant concentrations of all available prey items in a visited cell are summed together and lifetime exposure concentrations are calculated by averaging these cell-specific total contaminant concentrations of all cells visited, weighted for the time spent in each cell. Please refer to Loos *et al*. (2006) for more detail.

Risk indicator

Finally, risk indicators are also calculated similar to the cadmium case study following equation 5:

$$RI = \frac{PEC}{PNEC} \qquad (5)$$

RI = risk indicator (dimensionless)

PEC = predicted exposure concentration; lifetime averaged concentration in diet ($mg \cdot kg^{-1}$)

PNEC = predicted no-effect concentration (mg·kg-1)

PNEC values for nickel were calculated in the same way as PNEC values for cadmium, using a method developed by Traas *et al*. (1996; see also Loos *et al*. 2006 equation 18) to correct for differences in toxicity under laboratory and field conditions. No-effect concentrations (NOECs) were obtained from literature. Geometric mean NOECs where calculated for taxonomic classes (birds and mammals) and extrapolated to species-specific NOECs based on the corresponding species-specific diet compositions. Values used for the parameters in the methd of Traas *et al*. (1996) to calculate species-specific predicted no effect concentrations (PNECs) are given in Table 2.4.

Model validation for nickel accumulation

The predictive performance of the model was explored by comparing model predictions and measurements concerning internal nickel concentrations for four mammal species originating from the study area. Location-specific comparisons were facilitated by applying the capture locations of the animals as starting positions for the model simulations. Per starting position, 100 simulations were performed for each species. Species-specific averages and minimum and

maximum values were calculated for the internal nickel concentrations acquired in all cells visited in the total amount of simulations per capture location.

Table 2.4 (PNECs) *Input parameters for the calculation of predicted no effect concentrations in food*

Species	$NOEC_{food}$ ($mg \cdot kg^{-1}$)	EMR/FMR	FCC_{lab} ($kJ \cdot g^{-1}$)	FCC_{field} * ($kJ \cdot g^{-1}$)	FAE_{lab} **	FAE_{field}	PNEC *** ($mg \cdot kg^{-1}$ food)
Wood mouse	185.0 [1]	0,41 [3]	16.8 [3]	4.18	75.68	86.10 [3]	16.42
Bank vole	185.0 [1]	0,41 [3]	16.8 [3]	3.66	74.56	86.10 [3]	14.19
Common shrew	185.0 [1]	0,41 [3]	16.8 [3]	6.03	86.64	86.10 [3]	27.12
Common vole	185.0 [1]	0,41 [3]	16.8 [3]	3.93	74.00	86.10 [3]	15.10
European mole	185.0 [1]	0,41 [3]	16.8 [3]	3.84	88.00	86.10 [3]	17.55
Rabbit	185.0 [1]	0,41 [3]	16.8 [3]	3.93	74.00	86.10 [3]	15.10
Little owl	245.0 [2]	0,41 [3]	13.7 [3]	6.06	77.00	67.00 [4]	50.54
Common kestrel	245.0 [2]	0,41 [3]	13.7 [3]	7.10	84.00	67.00 [4]	64.66
Least weasel	185.0 [1]	0,41 [3]	16.8 [3]	7.07	85.01	86.10 [3]	31.20
Eurasian badger	185.0 [1]	0,41 [3]	16.8 [3]	4.91	84.02	86.10 [3]	21.43

* Calculated according to diet composition (see Loos et al. 2003 Appendix III) and mean caloric content of diet items (*Apodemus sylvaticus*, *Talpa europaea*, *Clethrionomys glareolus*, *Sorex araneus*, *Microtus arvalis*, *Oryctolagus cuniculus* 7.1 $kJ \cdot g^{-1}$; earthworms 3 $kJ \cdot g^{-1}$; hexapods & myriapods, isopods, spiders 7.2 $kJ \cdot g^{-1}$; gastropods 5.2 $kJ \cdot g^{-1}$ (Traas et al. 1996); vegetation 3.93 $kJ \cdot g^{-1}$; corn 14.48 $kJ \cdot g^{-1}$ (CSL 2002); fruits 1.92 $kJ \cdot g^{-1}$ (US-EPA 1993)

** Calculated according to diet composition and (see Loos et al. 2003 Appendix III) and predator/prey specific food assimilation efficiencies derived from CSL 2002.

*** Calculated according to Traas et al. 1996

1 = Geometric mean of reported NOECs for several mammalian species: Ambrose et al. 1976; O'Dell et al. 1970; Whanger 1973; 2 = Geometric mean of reported NOECs for several bird species: Cain & Pafford 1981; Weber & Reid 1968; 3 = Traas et al. 1996; 4 = CSL 2002

2.3.3 Ecological data

This study is applied to the same case study as the cadmium case study, i.e. the study area is identical as are the species simulated in this area. Therefore ecological data for this study is no different from the data used for the cadmium case as described in Loos et al. (2006). Please refer to this document for more details on ecological input data.

2.4 Cumulative exposure model

The SpaCE model is programmed in such a way that first the *landscape module* and the *foraging path module* are executed. The foraging paths of all individuals modelled are stored. Subsequently, the *exposure and risk module* is run for the first substance, using substance-specific input data and accumulation formulas. It calculates exposure to these individuals for all foraging paths stored. Subsequently the model is run for the next substance using the stored foraging paths, etc. Finally, when all substances are modelled, the *cumulative effects module* will be called for execution and risks will be calculated for cumulative exposure to these substances (see Appendix I a).

A joint action is defined as similar or dissimilar depending on whether the sites of primary action of the two chemicals are the same or different, and as interactive or non-interactive depending on whether one chemical does or does not influence the biological action of the other (Plackett

and Hewlett 1952). Problems can arise because different pairings can fall into different classes of joint action, and other joint actions may be possible between different pairs. Therefore, a mathematical description of the joint toxicity of a mixture of *n* compounds (*n*≥*2*) is possible only in a few cases, for which the absence of interaction seems a prerequisite (Van Leeuwen 1995). The mathematical descriptions of the two non-interactive actions, namely concentration addition or response addition are discussed in the following sections.

2.4.1 Concentration addition

The concept of concentration addition has been introduced by Loewe and Muischnek (1926, 1927) and is based on the idea that the compounds of a given mixture have a common site of action (Bliss 1939; Plackett and Hewlett 1952). Due to its reasonable pharmacological basis, concentration addition has gained large acceptance and has been proposed as the general solution for mixture toxicity analysis (Berenbaum 1985). Concentration addition is expressed mathematically as

$$E(c_{Mix})_{CA} = E(c_1 + ... + c_N)_{CA} = \sum_{i=1}^{n} E(c_i) = \sum_{i=1}^{n} \frac{C_i}{NOEC_i} \qquad (6)$$

where $E(c_{Mix})_{CA}$ denotes the risk indicator of an *n*-compound mixture, $NOEC_i$ is the no effect concentration of the i^{th} mixture component when applied singly and C_i is the concentration of the respective component in the mixture.

The predicted affected fraction (referred to as PAF) can then be calculated by running the model *x* times to simulate a population of *x* individuals and subsequently summing up the number of individuals exposed to levels above the toxicity reference value (TRV) and dividing this by the total number of individuals modelled.

2.4.2 Response addition

In contrast to concentration addition, the concept of response addition (also known as independent action or effect multiplication) is based on the assumption that the compounds of a given mixture act on different physiological systems within the exposed organisms (Bliss 1939). The mathematical formulation of response addition is as follows:

$$E(c_{Mix})_{RA} = E(c_1 + ... + c_N)_{RA} = 1 - \prod_{i=1}^{n} [1 - E(c_i)] \qquad (7)$$

where $E(c_{Mix})_{RA}$ denotes the predicted effect (scaled from 0–1) of an *n*-compound mixture, c_i is the concentration of the i^{th} compound, and $E(c_i)$ is the effect of that concentration if the compound is applied singly. The assumption may be stated explicitly in this form (Finney 1971; Harvey 1978; Stratton 1983) or in an equivalent form in which *E* is expressed as a fraction of unity and $P = 1 - E$ (Bliss 1939; Finney 1942, 1971; Webb 1963). For the two-agent case, this is

$$P(c_{1,2}) = P(c_1) + P(c_2) - P(c_1) \cdot P(c_2) \qquad (8)$$

The multiplication rule was originally derived from probability theory (Trevan 1927; Bliss 1939; Mather 1940; Finney 1942, 1971).

The PAFs for the single compounds, calculated in the same way as decribed in the concentration addition section, can be used to fill in equation 8 and will result in a PAF for the mixture.

Characterisation of cumulative risk to cadmium and nickel

The group of heavy metals can have many different modes of action on organisms depending on the properties of the heavy metal itself and on the test species (Roex *et al.* 2000). Cadmium

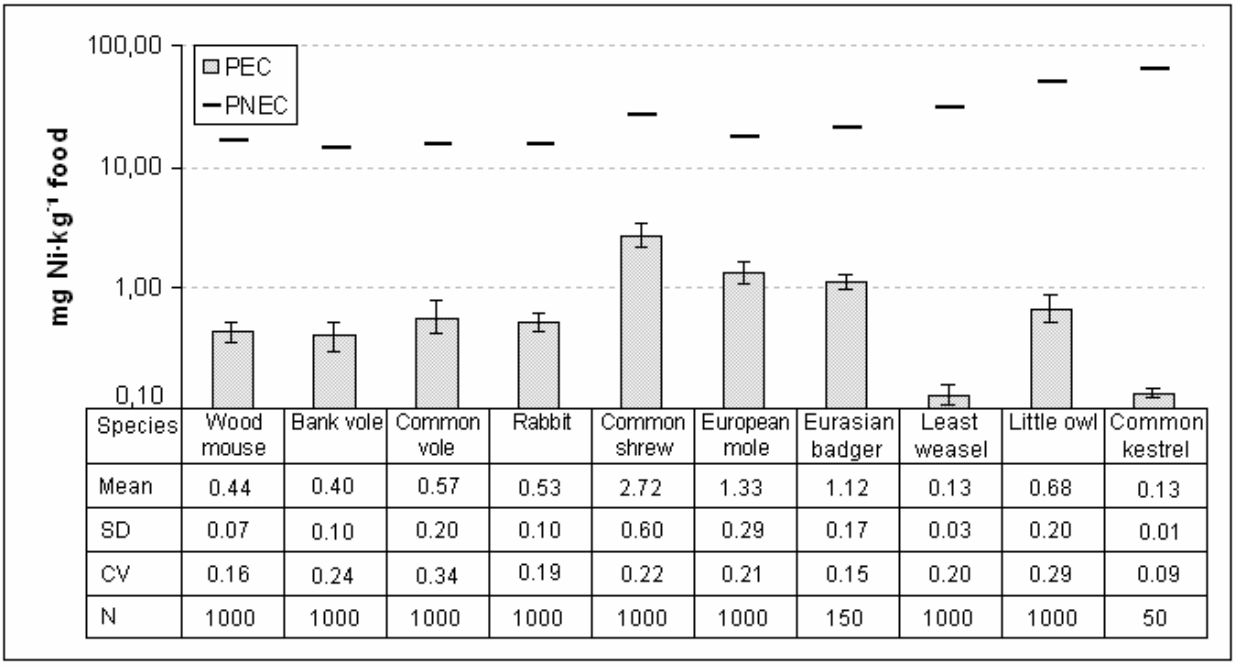
is toxic to a wide range of organs and tissues; however, the primary target organs of cadmium toxicity are the kidneys and liver (ATSDR 1999). Nickel may have several mechanisms governing toxicity. For example, the substitution of nickel for other essential elements may contribute to the adverse effects of nickel. Nickel can replace magnesium in certain steps in the activation of complement (McCoy and Kenney 1992). However, the mechanisms governing Ni^{2+} toxicity are not well understood (Schlicker 1999). This pleads to apply response addition for predicting cumulative risk to cadmium and nickel; it is unlikely that they act on exactly the same targets. However, eating or drinking levels of nickel much greater than the levels normally found in food and water have been reported to produce lung disease in dogs and rats and to affect the stomach, blood, liver, kidneys, and immune system in rats and mice, as well as their reproduction and development (ATSDR 2005). Therefore, cadmium and nickel might act upon the same organs, suggesting concentration addition, which can thus not be ruled out completely. Both response addition and concentration addition will be calculated to characterise joint action of cadmium and nickel. Although it is possible that the results for concentration addition are unrealistic in predicting the cumulative risk, they are at least useful for demonstration purposes and will be shown in the results section. Please refer to Appendix I b for VBA source code of the Cumulative Effects Module.

3 Results and discussion

3.1 Case study

For each of the 10 mobile vertebrate species selected, 1000 individuals were simulated to estimate their predicted exposure concentrations (PECs) for the ADW floodplain. These PECs were then compared with the predicted no effect concentrations (PNECs) to estimate the corresponding risk. Figure 3.1 shows the results of the simulations. These results illustrate that all species are exposed well below the toxicity reference value for nickel; they are not potentially at risk. Low PEC values may be expected, as nickel is an essential metal and vertebrates are expected to possess some ability to actively regulate the metal concentration internally. Phipps *et al*. (2002) state that Ni does not biomagnify in the terrestrial food web, suggesting that toxicity to higher trophic levels is unlikely.

Further, the PECs are considerably lower for the species Least weasel and Common kestrel, than for the other species and PECs for the Common shrew, the European mole and the Eurasian badger are among the higher ones. This tripartition is explicable, if we look at the species' diets. The Least weasel and Common kestrel have virtually no (relative highly accumulating) earthworms and vegetation in their food chain, whereas the Common shrew, the European mole and the Eurasian badger have a relatively high proportion of invertebrates in their diets.



Species	Wood mouse	Bank vole	Common vole	Rabbit	Common shrew	European mole	Eurasian badger	Least weasel	Little owl	Common kestrel
Mean	0.44	0.40	0.57	0.53	2.72	1.33	1.12	0.13	0.68	0.13
SD	0.07	0.10	0.20	0.10	0.60	0.29	0.17	0.03	0.20	0.01
CV	0.16	0.24	0.34	0.19	0.22	0.21	0.15	0.20	0.29	0.09
N	1000	1000	1000	1000	1000	1000	150	1000	1000	50

Figure 3.1 Predicted exposure concentrations (PECs) and predicted no-effect concentrations (PNECs) of nickel for vertebrate species in mg·kg^{-1} food. Error bars represent standard deviations. SD = standard deviation; CV = coefficient of variation; calculated as SD · mean^{-1}; N = number of model simulations. Note that a logarithmic scale is used.

Figure 3.2 shows the risk indicator estimations for multiple individuals of a single species set out in a frequency histogram. It illustrates that individuals of the Little owl species modelled are exposed to different levels of nickel in their food. If you compare this PEC distribution with the distribution of nickel soil concentrations into classes, there is a clear dissimilarity between the two (Figure 3.3). The former PEC distribution for multiple Little owl individuals is characterised by several peak exposures, while the latter contains only one peak. The peaks in Figure 3.2 coincide with different diet compositions that occur at different locations due to the variable availability of diet items at these locations. Besides the variability of nickel concentrations in the soil, the composition of the diet at different locations seems to play an important role in the actual exposure to a contaminant.

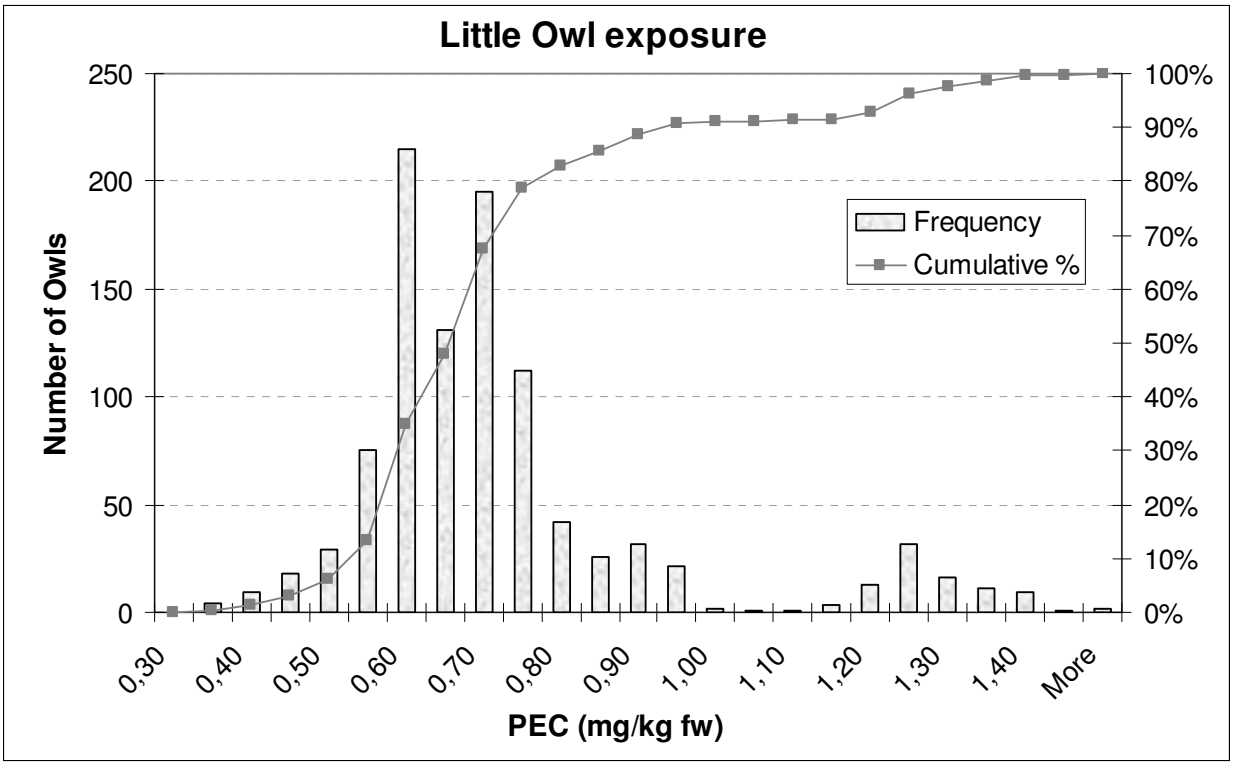


Figure 3.2 Frequency and cumulative percentage per nickel exposure concentration class predicted for 1000 individuals of the Little owl species modelled.

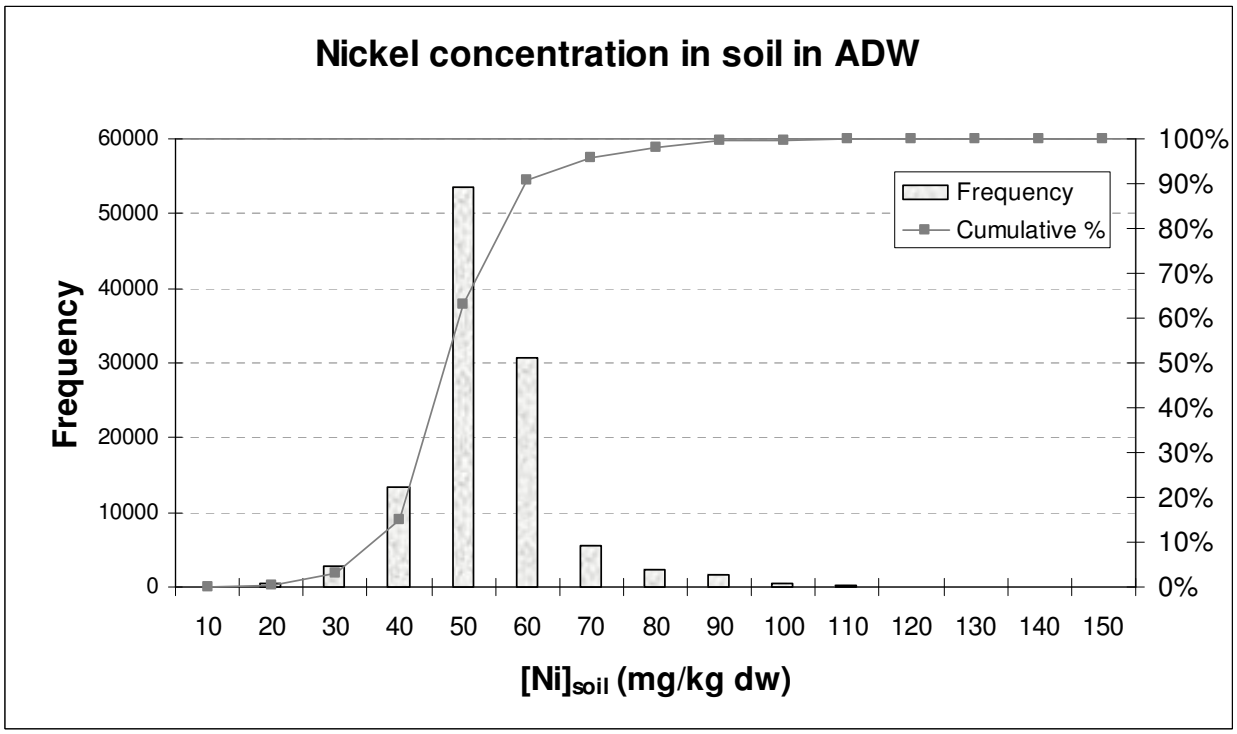
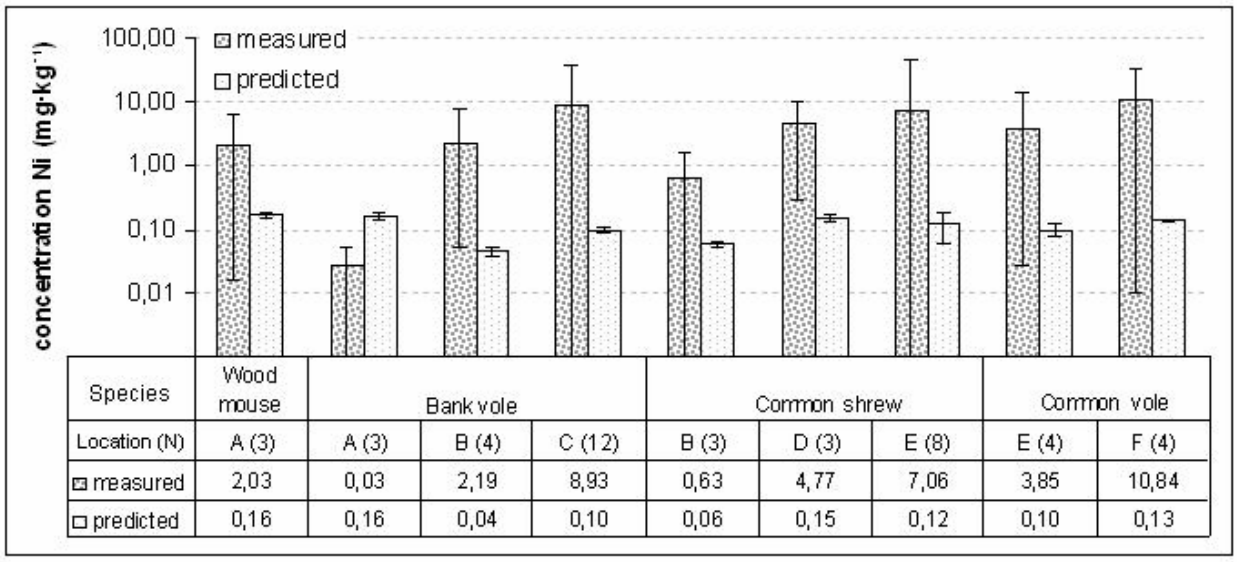


Figure 3.3 Frequency and cumulative percentage per nickel soil concentration class in the Afferdensche en Deestsche Waarden study area.

3.2 Model validation

For four small vertebrate species internal nickel concentrations predicted with the model were compared with measured concentrations of species captured in the study area. With the

exception of the Bank vole in location A, the model systematically underestimates the exposure to nickel (Figure 3.4). Measured and predicted internal nickel concentrations differ on average within a factor of 46, when excluding the Bank vole on location A. Relatively little research has been done on nickel accumulation, resulting in the fact that the nickel-specific input data for the model is based on the scarce data that could be found in literature. The BMF values used for predicting internal nickel concentrations in mice and shrew species are based on concentrations of species found in Bosveld *et al*. (2003) and Hendriks *et al*. (1995). These species had a considerable lower internal concentration (up to 25 times lower) than the species measured in the ADW study area, while the nickel concentrations in soil, in literature and in the study area, were comparable.



Species	Wood mouse	Bank vole			Common shrew			Common vole	
Location (N)	A (3)	A (3)	B (4)	C (12)	B (3)	D (3)	E (8)	E (4)	F (4)
measured	2,03	0,03	2,19	8,93	0,63	4,77	7,06	3,85	10,84
predicted	0,16	0,16	0,04	0,10	0,06	0,15	0,12	0,10	0,13

Figure 3.4 Location-specific measured and predicted internal nickel concentrations for four species. N indicates the number of individuals captured per location. Error bars indicate minimum and maximum nickel concentration values. Note that a logarithmic scale is used.

Figure 3.4 also shows that the individual variability of internal nickel concentrations in the various mice and shrew species (indicated by the error bars representing minimum and maximum values) are always lower for the species modelled than for the species captured in the field. The previous cadmium case (Loos *et al*. 2006) showed a similar trend; it indicates that spatial variation in environmental factors accounts for a minor part of the total variation observed in the field. Apparently, there are factors that are not included in the model, but which do account for a substantial proportion of the variation in internal concentration of nickel. Because nickel is an essential metal, active regulation of internal metal concentration in the individuals may cause part of the large variation observed in the field. Further, the biomagnification factors used in the model assume a constant ratio between contaminant concentration in food items and contaminant concentration in an organism, where the age of the receptors does not play any role. However, differences between individuals of different age classes might be of influence on the internal contaminant concentrations. For example, Kalisinska *et al*. (2004) found that the adult Szczecin Mallard muscles and brain were more nickel-rich than those of the immature individuals. Accordingly, it might be more appropriate to dynamically model nickel accumulation with formulas that take into account nickel absorption and excretion kinetics.

3.3 Mixture toxicity

For all vertebrate species modelled, risks to cadmium, nickel and a mixture of both have been calculated and species-specific affected fractions caused by these contaminants were predicted (Table 3.1). Assuming that the exposure estimates are realistic for both metals (see model validation section), cadmium poses the main hazard – where risk ranges from 0.13 to 11.00 – to the species. None of the species are at risk (ranging between 0.00 and 0.10) due to nickel

*Table 3.1 Risk and effect of cadmium, nickel, and a mixture of both metals predicted for the 10 vertebrate species modelled. Risk = mean risk indicator calculated with equation 6 for 1000 individuals (standard deviations between brackets); PAF = Predicted Affected Fraction of 1000 individuals modelled. CA = concentration addition; RA = response addition. *NB 50 and 150 individuals were modelled for the Kestrel and the Badger, respectively*

			Wood mouse	Bank vole	Common shrew	Common vole	European mole	Rabbit	Little owl	Common kestrel*	Least weasel	Eurasian badger*
single substance	Cd	Risk	0,49 (0,116)	0,47 (0,158)	2,58 (0,518)	0,43 (0,162)	9,13 (1,866)	0,35 (0,101)	11,00 (3,566)	0,13 (0,011)	3,40 (0,587)	5,82 (0,814)
		PAF	0,0%	0,0%	99,8%	0,9%	100,0%	0,0%	100,0%	0,0%	100,0%	100,0%
	Ni	Risk	0,03 (0,004)	0,03 (0,007)	0,10 (0,022)	0,04 (0,013)	0,08 (0,017)	0,04 (0,007)	0,01 (0,004)	0,00 (0,000)	0,00 (0,001)	0,05 (0,008)
		PAF	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%
mixture	CA	Risk	0,52 (0,118)	0,50 (0,163)	2,69 (0,535)	0,47 (0,173)	9,20 (1,875)	0,38 (0,105)	11,01 (3,564)	0,13 (0,011)	3,41 (0,588)	5,87 (0,817)
		PAF	0,0%	0,0%	99,8%	1,3%	100,0%	0,0%	100,0%	0,0%	100,0%	100,0%
	RA	PAF	0,0%	0,0%	99,8%	0,9%	100,0%	0,0%	100,0%	0,0%	100,0%	100,0%

exposure. PAFs calculated for the mixture with response addition are therefore similar to PAFs for cadmium only. However, if concentration addition is assumed, the predicted affected fraction of a species is higher for exposure to the mixture than exposure to cadmium alone. E.g. 0.9% of the Common voles are affected by cadmium, whereas the affected fraction increases to 1.3% when these voles are exposed to both cadmium and nickel. Except for the Little owl, standard deviations are always higher for the mixture than for the single substances.

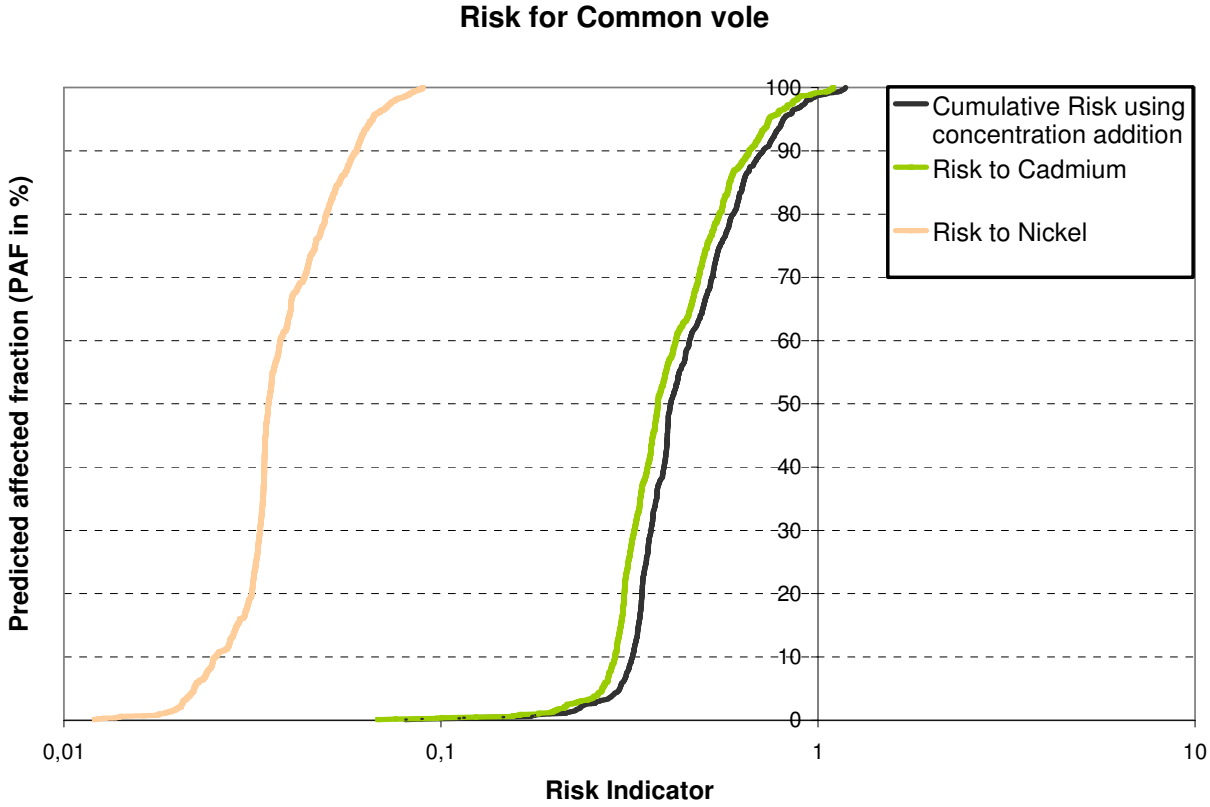


Figure 3.5 Risk indicator values predicted for 1000 individuals of the Common vole modelled of exposure to the heavy metals cadmium, nickel and to a mixture of both, calculated according to the concentration addition principle.

Figure 3.5 shows the predicted risks of 1000 Common voles presented in a cumulative distribution graph under the assumption of concentration additivity. This illustrates clearly how cumulative exposure to cadmium and nickel can produce an increase in the fraction of

individuals affected compared to exposure to the single substance, of which nickel solely does not affect any of the individuals (i.e. the black line –cumulative risk – is shifted to the right; more individuals are exposed to levels above the PNEC: RI > 1).

The model results show that for each individual a cumulative contaminant exposure can be calculated and that for each species the interindividual variability in exposure and risk to multiple stressors remains intact, due to the individual approach of the SpaCE model.

4 Conclusion

The Individual Based Exposure Model (IBEM) has been enhanced to model exposure to multiple stressors simultaneously and to calculate the cumulative effects using concentration addition and response addition principles. This new model is called Spatially explicit Cumulative Exposure model (SpaCE model). It simulates exposure to multiple substances in an efficient way. Firstly, it simulates and stores the spatial probability distribution of each of the moving individuals. Then it calculates the exposure of the individual receptors – using their stored spatial probability distributions – to each substance one after another. Finally, it calculates the cumulative exposure and effect. This approach leaves the individual-based and spatially explicit characteristics of the IBEM model intact. The SpaCE model contributes directly to one of the main NoMiracle objectives, i.e. to improve our understanding of complex exposure situations and develop adequate tools for exposure assessment, and, in particular, to explicitly address the spatial dimensions of cumulative risks.

The model was applied to a case study of cadmium and nickel exposure in the 'Afferdensche en Deestsche Waarden' floodplains (ADW). The results illustrated that all species are exposed well below the toxicity reference value for nickel, which hence does not pose a serious risk in the ADW. The interspecific differences in exposure to nickel can mainly be explained by the variations in diet preferences of the species. Comparison between predicted nickel exposure distribution for 1000 little owls and frequency distribution of nickel soil concentrations in the study area revealed that besides the nickel concentration in the soil the little owl's exposure is also influenced by the spatially variable availability of its diet items. Exposure to nickel was validated for four small vertebrate species in the study area. This proofed not to be very satisfactory; predictions were on average over 40 times lower than measurements. Literature about nickel accumulation in vertebrates was scarce and bioaccumulation factors may not be very appropriate in predicting accumulation of nickel. It is therefore recommended that the equations used for nickel accumulation need some revision, either by finding more complete and accurate parameter values for nickel or by using altered equations which incorporate factors that are important in determining the nickel exposure. For example, modelling nickel accumulation by explicitly taking into account absorption and excretion kinetics instead of using bioaccumulation factors could be an important improvement, since nickel is an essential metal and organisms are known capable of actively excreting nickel.

The cumulative effect results showed that cadmium was the main contributor causing adverse effects to the species modelled. If response addition is to be assumed for predicting cumulative effects for the mixture of cadmium and nickel, then nickel did not cause an added effect to any of the species. However, assuming concentration additivity, nickel exposure increased the predicted affected fraction of only the Common vole species with joint cadmium exposure. Cumulative exposure practically always increased the variability of the predicted risk compared to single exposure.

Although prediction of nickel exposure was not satisfactory, the SpaCE model is an adequate tool for predicting cumulative exposure in a spatially explicit and individual-based manner. The effects to multiple substances can be estimated for substances with either similar mode of action or dissimilar mode of action using two principles: concentration addition and response addition. This means that the model can only be used for predicting cumulative effect of substances that do not interact with each other.

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Appendices

Appendix I a Main Module

Option Explicit *'every variable must be declared. Otherwise, this condition will retrieve error.*

'----------

'Variables are defined here

'----------

Public Max_number_Individuals **As Integer** *'Number of modelled individuals/number of runs*

Public modelledSpecies **As Integer** *'type of species to model in these simulation runs*

Public Use_Soil_C **As Boolean** *'Choosing between Cd values for soil or sediment*

Public calc_sediment **As Boolean** *'option to calculate exposure using sediment concentrations.*

Public metalName **As String** *'calculate exposure for metal*

Public HQ_TV **As Single** *'This will be the minimum Hq_Total value a cell must have in order to individuals to move on/live in*

Public Organism(1 **To** 18) **As** organism_type *'organism 1 to 18 = earthworms to badger in "Basic_Data" WorkSheet.*

Public Organism_number **As Integer** *'organism code number*

Public Floodplain() **As** floodplain_**Type** *'reflects the characteristics of the floodplain.*

Public X_range **As Integer**, Y_range **As Integer** *'Number of cells in the x _axis and y_axis*

Public X **As Integer**, Y **As Integer** *'These variables will be used for searching the floodplain as coordinates*

Public Cell_area **As Integer** *'The area of the cell*

Public Foodchain_sheet(1 **To** 18, 1 **To** 8) **As** Foodchain_type *'reflects foodweb relationships. 1 - 20 are the predators and 1 to 8 are the preys.*

Public Species_ecotope_key(3 **To** 32, 0 **To** 18) **As Single** *'Is used to read the species_ecotope_key*

Public position() **As** position_type *' This will store information concerning position choosing*

Public Foraging_Path() **As** Foraging_Position_type *'this array will store information concerning the foraging positions*

Public Run **As Integer** *' Run number*

Public Preys_of_third_level() **As** Preys_third_level_type

Public Print_positions **As Boolean** *'Print foraging positions coordinates*

Public Print_position_concentration **As Boolean** *'Print Average Concentration in food and internal concentration acquired in each cell?*

Public Print_home_range_Hq_visits **As Boolean**

Public Print_final_results **As Boolean** *' Print Averages of all runs and risks*

Public Print_Hq **As Boolean** *'Print species-specific HQ_ecotope, HQ_food and HQ_total maps*

Public Print_PossibFirstPosition **As Boolean** *"Print species-specific possible first positions*

Public Moving_organism() **As** moving_organism_type *' Used to store Sum HQs of the foraged cells to each run and Maximum number of cell visited from all runs*

Public Position_number **As Long** *' Used as foraged position identification/counter*

Public Run_results() **As** run_results_type *' used to save the results of the runs*

Public counter **As Integer** *' Variable used to printing functions*

Public All_Runs_Results() **As** all_runs_results_type

Public Preys_Age_and_concentration_for_printing() **As** Preys_age_and_concentration_type

Public Age() **As Double**

```
Public max_number_positions As Long
Public home_range_cells() As position_type 'Will store the positions of the home range cells. It'll be used in FA calculations
Public Sample_Type As String
Public Home_range_cells_for_printing() As position_type
Public Cum_Run_Results() As cumu_run_results_type
Public Cum_All_Run_Risk_Indicator As Single
Public Cum_PAF_CA As Single 'PAF used for concentration addition

Public Type cumu_run_results_type

    risk_indicator As Single

End Type
Public Type Preys_age_and_concentration_type

    Age As Single
    concentration As Single

End Type

Public Type run_results_type

    X_coord As Integer 'x-coordinate of starting position (nest)
    Y_coord As Integer 'y-coordinate of starting position (nest)
    Time_weighted_average_concentration_food As Single ' Average concentration
    internal_concentration_acquired As Single ' Internal Concentration Acquired
    Time_weighted_Average_internal_concentration_acquired As Single
    risk_indicator As Single

End Type

Public Type all_runs_results_type

    Average_internal_concentration_acquired As Single
    Average_concentration_food As Single
    Average_Risk_Indicator As Single
    PAF As Single 'PAF used for single substances and response addition

End Type

Public Type moving_organism_type

    Sum_of_HQS As Single ' Sum of habitat Quality values for each run
```

Number_positions **As Long** *'Number of foraging positions*

number_home_range_cells **As Long**

End Type

Public Type Preys_third_level_type

internal_concentration **As Single** *'Potential Internal concentration for a Life Expectancy old animal*

Internal_concentration_Acquirable **As Single**

Q_number **As Integer** *' Prey identification*

relative_fraction **As Single**

End Type

Public Type floodplain_**Type** *'These are the variable of the array floodplain(), which reflects the caractheristics of the floodplain*

Ecotope **As Integer** *'This is the Ecotope number*

Hq_floodplain **As Single** *'This represents de HQ value that is read in the species ecotope key*

Hq_food **As Single** *'This represents the HQ related with food availability*

HQ_Total **As Single** *'This is the product of both other HQs*

First **As Boolean** *'This will determine if a cell can be a starting position*

Colonizable **As Boolean** *'This will determine if a cell is colonisable for organisms with a maximum dispersion distance (from unflooded areas)*

availability **As Boolean** *'This will retrieve whether or not a cell as any food items available*

Prey_sum **As Single** *' Sum of C,f and CAE's for each cell*

average_concentration_food **As Single** *' Average concentration in food for a second level species that forages there*

Available_Preys **As Integer** *' Number of preys available.*

Visited **As Boolean** *'This will reflect if a cell has been visited three selections before.*

Visits **As Integer** *'This is the number of times a cell has been visited*

Available_fractions **As Single** *'This will retrieve the sum of the available fractions.*

In **As Boolean** *'This will retrieve whether or not a cell is part of the floodplain*

Home_range **As Boolean** *'This will retrieve if a cell is within the home range*

Flooding_distances **As Single** *'Distance from unflooded cell*

internal_concentration **As Single**

End Type

Public Type position_type *'These are the variables of the array position(), which will store the positions retrieved by the random walk algorithm*

X_Pos **As Integer** *'X-coordinate*

Y_pos **As Integer** *'Y-coordinate*

number_of_visits **As Integer**

Cumulative_chance **As Single**

Distance **As Single**

Chance_index **As Single**

End Type

Public Type Foraging_Position_type *' Array containing Foraging positions*

X_Pos **As Integer** *'X-coordinate*

Y_pos **As Integer** *'Y-coordinate*

Age **As Single**

Time_spent **As Single** *' Number of days spent in cell foraged*

internal_concentration_acquired **As Single** *' Acquired internal concentration by foraging in that cell*

average_concentration_food **As Single** *' Average concentration in food of that cell*

Hq **As Single** *'Habitat Quality*

End Type

Public Type Foodchain_type *'These are the variables of the array foodchain_sheet, which reflects the foodchain relationships.*

BAF **As Single** *'Bioaccumulation Factor values*

Fractions **As Single** *'Fractions of diet*

Q_number **As Integer** *'Code number of the diet species*

Regression_a **As Single** *' regression coefficients*

Regression_b **As Single**

Regression_c **As Single**

Accumulation **As String**

BMF **As Single** *'Bio-magnification Factor values for level 2 organisms*

End Type

Public Type organism_type *'these are the variables of the array organism(), which will store individuals'charactheristics.*

Name **As String** * 20 *'Name*

Q_number **As Integer** *'Unique Identifying Code number for species*

Level_one **As Boolean**

Home_range **As Integer** *'Home Range*

Home_range_fraction **As Single** *'Percentage of Home Range in order to a cell to be used as a starting position*

Maximum_dispersion **As Integer** *'Maximum distance within which have to be in order to be colonized*

Max_hq **As Single** *'This is the variable that represents the maximum Hq value available on the floodplain for each species.*

number_of_startings **As Long**

Number_preys **As Integer** *'Number of preys*

```
    Life_expectancy As Integer
    DCR As Single 'Daily Consumption Factor (=Feeding Rate / Body Weight)
    BW As Single 'Body Weight
    CAE As Single 'Contaminant Assimilation Efficiency
    PNEC As Single 'Predicted No-Effect Concentration
    gutCorrectionFactor As Double 'Gut Content Correction Factor
    Number_of_individuals As Integer
    DMC As Single 'Dry Matter Content: dry weight to wet weight conversion factor (species specific)

End Type

Public Enum metals
    Total = 0
    Cd = 1
    Ni = 2
End Enum

Public metal As metals

Public Enum orgNumber
    soil = 0
    earthworms = 1
    Arachnida = 2
    Isopods = 3
    HM = 4
    Corn = 5
    vegetation = 6
    fruits = 7
    Gastropods = 8
    woodmouse = 9
    bank_vole = 10
    common_shrew = 11
    common_vole = 12
    mole = 13
    rabbit = 14
    little_owl = 15
    kestrel = 16
    weasel = 17
    badger = 18
End Enum
```

```
Sub model()
```

```
Dim max_number_preys

Debug.Print "start", Time
'-----------------------------------------------------------------------------------------------------------------------------
'Setting are defined here
'-----------------------------------------------------------------------------------------------------------------------------

Max_number_Individuals = Worksheets("basic_data").Cells(22, 12)
calc_sediment = False 'also make calculations with sediment concentrations?
Use_Soil_C = True 'If true soil values are used, (Note that IDW interpolated SoilConcentrations are used)
Sample_Type = "Soil" 'Note that IDW interpolated SoilConcentrations are used
Debug.Print RTrim(Sample_Type) + " Calculations"
metal = Cd 'ENTER element code
Call metalId

If metalName = "unknown" Then
    MsgBox "Error: unknown metal", _
                vbCritical, "RWMmessage"
    Exit Sub
End If

Print_positions = True
Print_position_concentration = True
Print_home_range_Hq_visits = True
Print_final_results = True
Print_Hq = False
Print_PossibFirstPosition = False
Cell_area = 25 'square meters
Y_range = 912 'cells
X_range = 245 'cells
HQ_TV = 0

ReDim Floodplain(soil To badger, 1 To Y_range, 1 To X_range) As floodplain_Type
Call Organisms

Dim simulateSpecies As Integer
simulateSpecies = 0

For Organism_number = woodmouse To badger
    If Organism(Organism_number).Number_of_individuals > 0 Then
        modelledSpecies = Organism_number
        simulateSpecies = simulateSpecies + 1
        End If
Next Organism_number
```

```
If simulateSpecies < 1 Then
        MsgBox "No individuals to simulate", _
                vbCritical, "RWMmessage"
        Exit Sub
ElseIf simulateSpecies > 1 Then
        MsgBox "Error: Multiple species input;  Which species do you wish to simulate?", _
                vbCritical, "RWMmessage"
        Exit Sub
 End If

Debug.Print "for organism: "; Organism(modelledSpecies).Name
ReDim home_range_cells(modelledSpecies To modelledSpecies, 1 To 1) As position_type
ReDim Home_range_cells_for_printing(1 To 5, modelledSpecies To modelledSpecies, 1 To 1) As position_type
ReDim Foraging_Path(1 To Max_number_Individuals, modelledSpecies To modelledSpecies, 1 To 1) As
Foraging_Position_type
ReDim Run_results(modelledSpecies To modelledSpecies, 1 To Max_number_Individuals , Total To Ni) As
run_results_type
ReDim All_Runs_Results(modelledSpecies To modelledSpecies, Total To Ni) As all_runs_results_type
ReDim Cum_Run_Results(1 To Max_number_Individuals) As cumu_run_results_type

If modelledSpecies > rabbit Then 'if a third-level species is being modelled
    max_number_preys = Organism(modelledSpecies).Number_preys
    ReDim Preys_of_third_level(little_owl To badger, 1 To Y_range, 1 To X_range, 1 To max_number_preys) As
    Preys_third_level_type
End If

Call virtual_floodplain
Call species_floodplain
Call Possible_First_Positions

Debug.Print "first Phase finished", Time

Call Foraging_Path_Procedure
ReDim Preys_Age_and_concentration_for_printing(1 To 5, earthworms To badger, 1 To max_number_positions) As
Preys_age_and_concentration_type
ReDim Age(earthworms To badger, 1 To max_number_positions) As Double

Debug.Print "exposure started", Time

Call Calculate_exposure

Debug.Print "Ready for Printing", Time

Call Write_in_Worksheets
```

```
'-----------------------------------------------

    If calc_sediment Then
        Use_Soil_C = False
        Sample_Type = "Sediment"
        Debug.Print RTrim(Sample_Type) + " Calculations"
        metal = Cd
        Call metalId
        Debug.Print "for organism: "; Organism(modelledSpecies).Name
        Print_home_range_Hq_visits = False

        Call species_floodplain
        Call Calculate_exposure
        Call Write_in_Worksheets
    End If
    '-----------------------------------------------

    Use_Soil_C = True
    Sample_Type = "Soil"
    Debug.Print RTrim(Sample_Type) + " Calculations"
    metal = Ni
    Call metalId
    Debug.Print "for organism: "; Organism(modelledSpecies).Name
    Print_home_range_Hq_visits = False

    Call Organisms
    Call species_floodplain
    Call Calculate_exposure
    Call Write_in_Worksheets
    '-----------------------------------------------

    If calc_sediment Then
        metal = Ni
        Call metalId
        Use_Soil_C = True
        Sample_Type = "Sediment"
        Debug.Print RTrim(Sample_Type) + " Calculations"
        Debug.Print "for metal: "; metalName
        Debug.Print "for organism: "; Organism(modelledSpecies).Name
        Print_home_range_Hq_visits = False

        Call species_floodplain
        Call Calculate_exposure
```

```
    Call Write_in_Worksheets
  End If

  Call Cumulative_effect

  Debug.Print "finished", Time
  Debug.Print

End Sub
```

```
Sub metalId()

  If metal = Cd Then
    metalName = "Cd"
    Debug.Print "for metal: "; metalName
  ElseIf metal = Ni Then
    metalName = "Ni"
    Debug.Print "for metal: "; metalName
  Else
      metalName = "unknown"
  End If

End Sub
```

Appendix I b Cumulative Effects Module

```
Sub Cumulative_effect()

Debug.Print "Start Cumulative Module"

Dim Cum_Run_Risk_Indicator As Single
Dim Sum_Cum_Run_Risk_Indicator As Single
Dim Cum_affected_individuals As Integer

Cum_Run_Risk_Indicator = 0
Sum_Cum_Run_Risk_Indicator = 0
Cum_All_Run_Risk_Indicator = 0
Cum_affected_individuals = 0

For Organism_number = modelledSpecies To modelledSpecies
    ''''''''''''''''''''''''''''''''''''''''''''''''''''''''''
      'Concentration addition:
      ''''''''''''''''''''''''''''''''''''''''''''''''''''''''''
      For Run = 1 To Max_number_Individuals
        Cum_Run_Risk_Indicator = Run_results(Organism_number, Run, Cd).risk_indicator +
        Run_results(Organism_number, Run, Ni).risk_indicator
        Cum_Run_Results(Run).risk_indicator = Cum_Run_Risk_Indicator
        Sum_Cum_Run_Risk_Indicator = Sum_Cum_Run_Risk_Indicator + Cum_Run_Risk_Indicator
              If Cum_Run_Results(Run).risk_indicator >= 1 Then
                    Cum_affected_individuals = Cum_affected_individuals + 1
                    End If
      Next Run
    Cum_All_Run_Risk_Indicator = Sum_Cum_Run_Risk_Indicator /
    Organism(Organism_number).Number_of_individuals
    Cum_PAF_CA = Cum_affected_individuals / Organism(Organism_number).Number_of_individuals
      ''''''''''''''''''''''''''''''''''''''''''''''''''''''''''
      'Response addition:
      ''''''''''''''''''''''''''''''''''''''''''''''''''''''''''
    All_Runs_Results(Organism_number, Total).PAF = 1 - (1 - All_Runs_Results(Organism_number, Cd).PAF) * (1
    - All_Runs_Results(Organism_number, Ni).PAF)
Next Organism_number

Call Print_Cumulative_Results

End Sub
```